

## ABSTRACT

All *Borrelia burgdorferi sensu lato* isolates characterized to date have one or a combination of several major outer surface proteins (Osp). Mutants of *B. burgdorferi* lacking Osp proteins were selected with polyclonal or monoclonal antibodies at a frequency of  $10^{-6}$  to  $10^{-5}$ . One mutant that lacked OspA, B, C and D was further characterized in the present study. It was distinguished from the OspA<sup>+</sup>B<sup>+</sup> cells by its (i) auto-aggregation and slower growth rate, (ii) decreased plating efficiency on solid medium, (iii) serum- and complement-sensitivity, and (iv) diminished capacity to adhere to human umbilical vein endothelial cells. The Osp-less mutant was unable to evoke a detectable immune response after intradermal live cell immunization even though mutant survived in the skin the same duration as wild-type cells. Polyclonal mouse serum raised against Osp-less cells inhibited growth of the mutant but not of wild-type cells, an indication that other antigens are present on the surface of the Osp-less mutant. Two different classes, A and B, of monoclonal antibodies (mAb) with growth inhibiting properties for mutant cells were produced. Class A mAbs bound to 13 kDa surface proteins of *B. burgdorferi sensu stricto* and of *B. afzelii*. The minimum inhibitory concentration of the Fab fragment of one mAb of this class was 0.2 µg/ml. Class B mAbs did not bind by Western Blot to *B. burgdorferi* cells but reacted with cells in an unfixed cell immunofluorescence assay and growth inhibition assay. These studies revealed hitherto unknown functional aspects of Osp proteins, notably serum-resistance, and indicated that in the absence of Osp proteins other antigens are expressed or become accessible at the cell's surface.